Project Report: Molecular Analysis of Microbial Ecosystems in Extreme Enviornments

Lead Team:	***University of Colorado, Boulder***
Project Title:	***Molecular Analysis of Microbial Ecosystems in Extreme Enviornments***
Project Investigator:	***Norman Pace***

Project Progress

Projects continue to revolve around the development and use of ribosomal ribonucleic acid (rRNA) based molecular methods to survey and study the microbial constituents of ecosystems in extreme environments without the requirement for cultivation of the organisms. This cultivation−independent approach to ecosystems analysis is essential because most microbes, >99%, cannot be cultured using standard techniques. With the molecular methods, rRNA genes are cloned directly from environmental DNA, and then sequenced to gain a phylogenetic snapshot of the organisms represented by the cloned genes. Some properties of organisms can be inferred from the phylogenetic results, and the sequences can be used to design hybridization probes to visualize organisms and their interactions in the environment. NAI sponsored studies include:

- Antarctic and Colorado endolithic communities. Primary productivity in rocks occurs through the action of endolithic microbial communities (photosynthesis−driven communities in the outer few cm of any rock surface exposed to light). These communities so far have received only limited study, and only with classic microscopy and culture techniques. Ongoing rRNA gene analyses of two selected communities from Antarctica (Collaboration with Imre Friedman) and four rock types from Colorado have revealed many novel kinds of organisms, some closely related to described organisms but others very different. Although previously considered dominated by cyanobacteria, an abundance of chloroplast sequences has been detected. The nature of the eucaryal component expected is not yet known. Remarkably, an abundance of representatives of the Thermus/Deinococcus division of Bacteria has been detected that were previously unknown, but are related to the “radiation resistant” deinococci. These new organisms also may predictably be similarly robust; the “radiation resistant” property of the deinococci is now thought to be protection against dessication/oxidative damage.

- Yellowstone high−temperature settings: This laboratory has for many years studied thermophilic ecosystems at Yellowstone and elsewhere. Current activities continue to explore the makeup of properties of communities driven by hydrogen−metabolism, probably the dominant form of primary productivity in high−temperature settings anywhere. Immediate goals include the detailed mapping of recently recognized, (probably) hydrogen−supported stromatolite−like structures in Yellowstone's Obsidian Pool.
- Anaerobic environmental eucaryotes. We have continued an rRNA−based survey of eucaryal phylotypes in anaerobic settings, for instance, anaerobic marine and freshwater sediments. Recent results have identified a wealth of novel eucaryotic microbial diversity, including eight (!) novel kingdom−level clades, some among the most deeply divergent of eucaryal rRNA sequences. Current efforts focus on hypersaline ecosystems, mainly Guerrero Negro.
- We have begun extensive analysis of the microbial composition of Guerrero Negro hypersaline mats, in concert with geochemical and other biological studies of the NAI Ecogenomics group. Particularly noteworthy at this stage is the finding, based on rRNA gene abundance, that so−called Green Nonsulfur Bacteria, not cyanoacteria, are the dominant species. This poses many hypotheses and new questions.

Overall, we are making excellent progress and this work is conspicuous.

Highlights

- Eight novel, kingdom−level phylogenetic groups of eukaryotes were discovered (published) in anoxic ecosystems, including forms that branch deeply in evolutionary trees.

- The dominant organisms in Yellowstone high−temperature settings are hydrogen−metabolizers, and we have now measued hydrogen in several Yellowstone hotsprings.

- Hydrogen, not sulfur, is the driving energy source of this and probably other geothermal ecosystems.

- Based on Guerrero Negro studies so far, Green Nonsulfur Bacteria, not cyanobacteria, dominate hypersaline microbial mats. This finding brings to question the traditional shibboleth that cyanobacteria are the leading producers in such ecosystems.

Roadmap Objectives

- ***Objective No. 2:*** Origin of Life?s Cellular Components
- ***Objective No. 3:*** Models for Life
- ***Objective No. 4:*** Genomic Clues to Evolution
- ***Objective No. 5:*** Linking Planetary Biological Evolution
- ***Objective No. 6:*** Microbial Ecology
- ***Objective No. 7:*** Extremes of Life

- ***Objective No. 10:*** Natural Migration of Life

Field Expeditions

Field Trip Name: Baja Field Trip	
Start Date: 05/30/2001	***End Date:*** 06/06/2001
Continent: North America	***Country:*** Mexico
State/Province: Baja California, Sur	***Nearest City/Town:*** Guerrero Negro
Latitude:	***Longitude:***
Name of site(cave, mine, e.g.): Below	***Keywords:***
Description of Work: Collection of Microbial Mat samples for molecular analysis from the solar evaporation ponds of the Exportadora de Sal.	
Members Involved: Ruth Ley, Norman Pace and John Spear.	

Field Trip Name: Baja Field Trip	
Start Date: 10/13/2001	***End Date:*** 10/16/2001
Continent: North America	***Country:*** Mexico
State/Province: Baja California Sur	***Nearest City/Town:*** Guerrero Negro
Latitude:	***Longitude:***
Name of site(cave, mine, e.g.): Below	***Keywords:***
Description of Work: Collection of Microbial Mat samples for molecular analysis from the solar evaporation ponds of the Exportadora de Sal.	
Members Involved: Ruth Ley and John Spear	

Field Trip Name: Yellowstone Field Trip	
Start Date: 06/25/2001	***End Date:*** 06/28/2001
Continent: North America	***Country:*** USA
State/Province: Wyoming	***Nearest City/Town:*** Jackson, Wyoming
Latitude:	***Longitude:***
Name of site(cave, mine, e.g.): Below	***Keywords:***
Description of Work: Collection of samples from hot spring pools for	

molecular analysis. Analysis of hydrogen concentrations in hot springs for comparison to microbial mats from Guerrero Negro, Baja California, Sur.
Members Involved: John Spear and Jeff Walker

Field Trip Name: Kamchatka Peninsula	
Start Date: 08/10/2001	***End Date:*** 08/20/2001
Continent: Eurasia	***Country:*** Russia
State/Province: Kamchatka	***Nearest City/Town:*** Petropavlosk
Latitude: 53 N	***Longitude:*** 158.5 W
Name of site(cave, mine, e.g.): Below	***Keywords:***
Description of Work: Investigation of thermal features around the Kamchatka peninsula for collaborations by Russian and US teams within the Astrobiology program.	
Members Involved: John Spear	

Field Trip Name: Yellowstone Field Trip	
Start Date: 10/07/2001	***End Date:*** 10/11/2001
Continent: North America	***Country:*** USA
State/Province: Wyoming	***Nearest City/Town:*** Jackson, Wyoming
Latitude:	***Longitude:***
Name of site(cave, mine, e.g.): Below	***Keywords:***
Description of Work: Collection of samples from hot spring pools for molecular analysis. Analysis of hydrogen concentrations in hot springs for comparison to microbial mats from Guerrero Negro, Baja California, Sur.	
Members Involved: John Spear and Jeff Walker	

Cross Team Collaborations

We are actively engaged with other NAI groups involved in the Ecogenomics team − MBL/Harvard, NASA Ames, Arizona − in a comprehensive analysis of the Guerrero Negro hypersaline microbial mat ecosystem. An early outcome of the study from our perspective is the discovery of the prevalent

Green−Nonsulfur Bacteria. Work from other teams will be important in corroborating that finding.